Characterization of Infant Mu Rhythm Immediately before Crawling: A High-Resolution EEG Study

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Abstract

Crawling is an important milestone in infant motor development. However, infants with developmental motor disorders can exhibit delays, or even miss, in the acquisition of crawling skill. And little information is available from the neurodevelopmental domain about the changes in brain function with intervention. The mu rhythm can potentially play a substantial role in understanding human motor development at early ages in infants, as it has in adults. Studies about the mu rhythm in infants were in coarse temporal resolution with longitudinal samples taken months or years apart. Details about the infant mu rhythm at a fine age resolution has not
been fully revealed, which leads to contradictory evidence about its formulation and developmental changes of its spectral origins and, therefore, impedes the full understanding of motor brain development before crawling skill acquisition. The present study aims to expand knowledge about the infant mu rhythm and its spatio-spectral pattern shifts along maturation immediately before crawling. With high-density EEG data recorded on a weekly basis and simultaneous characterization of spatio-spectral patterns of the mu rhythm, subtle developmental changes in its spectral peak, frequency range, and scalp topography are revealed. This mu rhythm further indicates a significant correlation to the crawling onset while powers from other frequency bands do not show such correlations. These details of developmental changes about the mu rhythm provide an insight of rapid changes in the human motor cortex in the first year of life. Our results are consistent with previous findings about the peak frequency shifting of the mu rhythm and further depict detailed developmental curves of its frequency ranges and spatial topographies. The infant mu rhythm could potentially be used to assess motor brain deficiencies at early ages and to evaluate intervention effectiveness in children with neuromotor disorders.

Keywords
Mu rhythm; Infant EEG; Motor development; High resolution; Crawling

1. Introduction
Crawling is a form of prone locomotion available to infants during their first year of life, and its onset is an important milestone in infant development. Although affected by various factors, such as body dimensions and experience (Adolph et al., 1998), most infants learn to crawl in the second half year of age, with the full acquisition around 8 or 9 months of age (Centers for Disease Control and Prevention, CDC, 2015). Crawling further exhibits close
relations to the emergence and development of many neuropsychological behaviors. For example, the ability to crawl enables the learning of coordinated limb and joint movement that serve as the building blocks for the acquisition of later motor-related functions, including cruising and walking (Adolph et al., 2011). Crawling has also been found to correlate with cognitive development, e.g., spatial memory (Kermoian and Campos, 1988; Clearfield, 2004; Clearfield et al., 2008), and socio-emotional behavior, e.g., differentiated emotional responses and height awareness (Richards and Rader, 1983; Campos et al., 1992).

The process of crawling skill acquisition can be interrupted by neurological disorders such as cerebral palsy (CP) (Jones et al., 2007). Infants with CP usually experience delay or lack of crawling skill acquisition, which not only affects motor skill development, but also leads to deficiency in cognitive development (Bottcher, 2010). Both brain plasticity theory (Bach-y-Rita, 1990) and neuronal group selection theory (Edelman, 1993; Hadders-Algra, 2000) suggest that, with intervention and training at early ages, better modulation and coordination of movements can be achieved. A few studies have also demonstrated an association of early interventions with positive effects on motor development (Blauw-Hospers and Hadders-Algra, 2005). In particular, recently developed robotic assistants (Kolobe et al., 2013; Miller et al., 2015) show promising efficacy in promoting crawling movement at early ages. However, changes in the neurodevelopmental domain are less known suggesting the need to understand neural biomarkers of infant motor development, especially during the period for crawling skill acquisition.

In adults, the mu rhythm in electroencephalography (EEG) is a widely investigated motor brain biomarker due to its correlation to human motor functionalities (Pfurscheller and Lopes da Silva, 1999; Pfurtscheller et al., 2006; Yuan and He, 2014). It represents oscillatory activities in the alpha band (8-12 Hz) over the central areas of the human brain. The mu rhythm is prominent
during the resting state, and attenuated during the preparation and execution of voluntary movement (Neuper et al., 2006) or the production of motor imagery (Pfurscheller et al., 2006) at the contralateral motor cortex. It is accompanied by a harmonic decrease in the beta band (13-30 Hz) (Pfurscheller and Lopes da Silva, 1999). The emergence and attenuation of the mu rhythm in adults are induced by event-related synchronization (ERS) and event-related desynchronization (ERD) in the neuronal activity (Pfurscheller and Lopes da Silva, 1999), which reflect sensorimotor deactivation and activation (Neuper et al., 2006), respectively.

Compared with adult mu rhythm studies, few studies of mu rhythm have been done with infants. The major findings in previous infant EEG studies are: 1) the range of alpha band (the frequency band where the mu rhythm resides) to be lower than that of adults (Smith, 1938; Lindsley, 1939; Marshal et al., 2002; Orekhova et al., 2006) and 2) its continuous increase with maturation toward the alpha range exhibited by adults (Cuevas et al., 2014). An alpha rhythm originated over the central cortical site is considered as the infant mu rhythm, due to its functional resemblance with the adult mu rhythm (Stroganove et al., 1999). As early as 1939, Smith reported a transition of sensorimotor EEG oscillations of 7 Hz at birth to 8.5 Hz at 18 months of age. Hagne et al. (1973) found the emergence of the mu rhythm peaks at 6 months of age and the shift of its peak frequency from 6 Hz at 6 months to 7 Hz at 12 months of age. Marshal et al. (2002) identified the emergence of a 7-8 Hz mu rhythm peak at 10 months of age, shifting towards 8 Hz at 14 and 24 months of age. Orekhova et al. (2006) found the mu rhythm ranging from 6.4-8.4 Hz for infants about 10 months of age to 8.4-10.4 Hz for 5-year-old children. Berchicci et al. (2011) reported the shift of mu rhythm peaks from 2.75 Hz at as early as 3 months to 8.25 Hz at 11 months of age in magnetoencephalography (MEG) data. Besides these developmental changes, the mu rhythm is also found to be associated with the level of
motor experience. For infants who are learning to crawl, their mu rhythms present stronger suppression in 7-9 Hz when they observe video of crawling than video of walking, which is an unfamiliar motor skill to them (van Elk et al., 2008).

There are inconsistencies in the findings about the infant mu rhythm in terms of both its time of emergence and its range of frequencies. While most studies reported the timing of infant mu rhythm emergence not until several months of age (usually between 5 and 8 months) (Hagne et al., 1973; Marshal et al., 2002), Smith (1939) reported the existence of mu rhythm of 7 Hz at birth. While most studies reported the frequency range of 6-9 Hz for mu rhythm at emergence around 5 to 8 months (Stroganove et al., 1999; Orekhova et al., 2006; Marshal et al., 2002), Berchicci et al. (2011) argued that it starts as low as 2.75 Hz at 3 months of age. Although some of the early studies might have been constrained by available technologies at that time, it is more reasonable to attribute these inconsistencies to limited simultaneous investigation of spatio-spectral patterns along with their developmental changes from maturation. Characterization of the infant mu rhythm in these studies was mostly conducted on pre-selected channels based on \textit{a priori} assumptions and its spatial patterns have not been sufficiently studied with high-density spatial samplings. Moreover, considering developmental changes, many previous studies were cross-sectional studies that used different subjects at different age points and, therefore, lacked the information of cause and effect in the mu rhythm development (Stroganove et al., 1999; Orekhova et al., 2006). A few longitudinal studies were conducted with a coarse age resolution, usually months (Stroganove et al., 1999) or years between adjacent age points (Marshal et al., 2002). Detailed developmental changes of the infant mu rhythm with a fine age resolution cannot be thus captured. This resolution in the mu rhythm is needed, in particular, to reveal subtle neural developmental changes behind important milestones in motor development, e.g., acquisition of
crawling.

In the present study, detailed developmental information about the infant mu rhythm is investigated at a fine age resolution from weekly recorded EEG data in infants of 5 to 7 months of age, a critical period for crawling skill acquisition. Age-related changes in characteristics of the infant mu rhythm, including peak formulation, peak shifting, and frequency range, are studied. Moreover, spatial patterns of the infant mu rhythm are evaluated with high-density EEG data (i.e., 124 channels). The correlation between identified mu rhythm power and crawling onset time is also analyzed to understand behavioral relevance of the mu rhythm. The experimental results demonstrate the formulation of mu rhythm peak around 6 months of age, and the shift of its spectral range and peak towards higher frequency during this age range. High-resolution spectral topographies reveal substantial activation of the infant mu rhythm in the motor cortex, its asynchronous developmental changes among different sites within the motor cortex, and its significant correlation to crawling skill acquisition. These findings provide data about detailed developmental changes of the infant mu rhythm with high spatial and temporal resolutions, as well as behavioral relevance, which are not available in previous studies and are a contribution to the collected knowledge of infant motor development.

2. Method

2.1 Participants

Fifteen infants participated in the study, with informed consents obtained from their parents. Data from two participants were excluded from the further analysis because one was diagnosed with atypical development in the post-experiment evaluation and another one had only limited age overlap with other infants after gestation age adjustment (7-9 months). The other thirteen participants were all typically developing infants (8 males, 5 females), with their
gestation adjusted age ranging from 17 to 23 weeks (mean: 20.54 weeks; SD: 1.45 weeks) at the time of their first EEG recording. The study was reviewed and approved by the institutional review board at the University of Oklahoma Health Sciences Center (IRB number 3755).

2.2 Data acquisition

EEG data were acquired with the EGI’s Geodesic EEG System 300 (Electrical Geodesics, Inc., Eugene, OR), including a Net Amps 300 amplifier and a 124-channel HydroCel Geodesic Sensor Net (HCGSN 130) for infants. EEG sensor nets with three different sizes (40-42 cm, 42-43 cm, and 43-44 cm) were used for infants of various head circumferences. During data collection, infants wore EEG sensor nets and were seated on their parents’ laps. Parents were instructed to avoid rocking and/or moving infants, and to keep their infants’ heads upright. A baby rattle app on cellphone was presented to infants, out of their reaching range (approximately 1 meter away), to keep them calm and still during EEG recording. Resting EEG data were recorded at a sampling frequency of 1 kHz for 5 minutes in each experimental session in a room without shielding.

Aside from EEG data, videos were recorded simultaneously in each session, synchronized to EEG data through the EGI’s Netstation software. Participants attended weekly EEG recording sessions until 8 months of age or they were able to crawl. Crawling onset dates were reported based on either health professionals’ observation during experimental sessions or parents’ report during follow-up phone interviews. Data recorded in sessions between 5 and 7 months of age were selected for further analysis since only few sessions were available at 8 and 9 months of age from all participants, as shown in Table 1. Sessions with excessive motion, identified by visual inspections of video and EEG recordings, were also excluded. There were, on average, ten EEG sessions included from each participant after implementing these exclusion
criteria, except one with only 5 sessions (the participant was able to crawl at the beginning of 7 months, and graduated from the study at that time). A full list of participants and sessions included in the analysis at various months of age is provided in Table 1.

**Table 1** Numbers of participants and sessions included in analysis at various months of age.

<table>
<thead>
<tr>
<th>Gestation adjusted age</th>
<th>Month 5</th>
<th>Month 6</th>
<th>Month 7</th>
<th>Month 8</th>
<th>Month 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sessions</td>
<td>37</td>
<td>43</td>
<td>37</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Number of participants</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

2.3 Preprocessing of EEG data

The following preprocessing steps were implemented for EEG artifact rejection using the EEGLAB toolbox (Delorme and Makeig, 2004), which were specifically tailored to deal with noisy EEG data acquired from a group of participants who were difficult to control for such recordings and were in a room without shielding. First, a 0.3-30 Hz band-pass digital filter was used on the EEG data from all sessions to remove DC offsets and undesired high-frequency interference, e.g., muscle activity and power line noise. Second, EEG data from channels on the boundary of the sensor net (red ones in the left insert of Figure 1(a)), i.e., those near the neck, ears and eyes, were excluded since they were heavily susceptible to head, face and eye movements. This step left 70 channels (green and black ones in the left insert of Figure 1(a)) on the top of infant heads for further analysis. Third, EEG segments with abnormally large amplitudes from visual inspection of EEG waveform and those corresponding to abrupt voluntary movements in video recordings were marked and removed. After the bad segment rejection, there were, in general, two minutes of EEG data left in each session for each participant. Fourth, bad EEG channels were identified when their kurtosis statistic was larger
than 5 times of the standard deviation of data from the 70 channels, and interpolated using data from neighboring channels. Then, the EEG data were re-referenced to the common average reference calculated from all channels. Last, a blind source decomposition method, i.e., independent component analysis (ICA), was implemented to detect EEG components generated by sources other than neuronal ones (Delorme et al., 2012). EEG data were decomposed into 30 independent components (IC), and those ICs associated with common EEG artifacts, including eye movements, heart activities, muscular activities, etc., were visually identified and removed.

2.4 Spectral power analysis of EEG data

For each EEG channel, power spectral densities (PSD) were estimated based on Welch’s method (Welch, 1967) implemented in the “pwelch” function of the Matlab software (R2014a, MathWorks Inc., Natick, MA). This method estimated the spectral power in three-second Hanning-windowed epochs that had been segmented from the continuous EEG data. Adjacent epochs overlapped by 50%. This resulted in a frequency resolution of 1/3 Hz in the PSDs. Following Marton et al. (2014), relative power spectral density for each frequency bin between 1 and 30 Hz with 1/3 Hz increment was computed by normalizing the absolute PSD estimates:

\[
R_f = \frac{P_f}{\sum_{f=1}^{30} P_f}, \quad f = 1, \frac{1}{3}, \frac{2}{3}, 2, \ldots, 30 \text{ Hz},
\]

where \(R_f\) and \(P_f\) are relative and absolute PSD at each frequency bin \(f\), respectively. This step is to minimize the influence of EEG PSD variations in different participants and recording sessions (Marton et al., 2014).

Relative PSDs were registered to their corresponding geodesic locations on the scalp to generate longitudinal topographies for each frequency bin. To facilitate the spatio-spectral analysis (see Section 2.6), unequal EEG PSD magnitudes at different frequencies, also known as the power-law decay, have been addressed. Therefore, relative PSDs on each channel were
normalized to obtain Z scores:

\[ Z_f = \frac{R_f - \mu_{R_f}}{\sigma_{R_f}}, \quad f = 1, \frac{1}{3}, \frac{2}{3}, 2, \ldots, 30 \text{ Hz}, \]  

(2)

where \( Z_f \) is the Z score at the frequency bin \( f \) after normalization; \( \mu_{R_f} \) and \( \sigma_{R_f} \) are the mean and standard deviation of relative PSDs at the frequency bin \( f \) across all channels, respectively.

2.5 Spectral Peak Statistics

Spectral peak has been identified as an important characteristic in evaluating mu rhythm development (Marshall et al., 2002). In the present study, spectral peaks were identified on single-trial PSDs from each participant and age point, and spectral peak statistics at different months as functions of frequency were constructed. A spectral peak in a single-trial PSD estimation was determined by its relative PSD data at any frequency bin in frequency range of 2-9 Hz (the range covering most significant rhythmic activities in infant EEG PSDs), which was larger than those of its two closest frequency bins on each side on channels in the central cortex covering the motor area (black ones in the left insert of Figure 1(a)). Spectral peak distribution as a function of frequency was constructed by summing the number of spectral peaks at each frequency bin from all central channels and sessions at each age point, which was normalized by dividing the corresponding numbers of sessions since they were different at different age points. Following that, Gaussian curve fitting was performed on spectral peak distribution data between 5 and 9 Hz (assumed alpha band, see details in section 2.6 below) to quantify peak frequencies of the alpha band at different months.

2.6 Clustering analysis of spatio-spectral patterns

While the definitions of the delta (below 4 Hz), theta (about 4-6 Hz) and alpha bands (about 6-9 Hz) have been adopted in many infant EEG studies (Stroganova et al., 1999;
Orekhova et al., 2006; Saby and Marshall, 2012), transitions of these frequency bands in infants toward adults (Cuevas et al., 2014) and peak frequency shifts as suggested in infant studies otherwise indicate the progression of spatial and spectral patterns of the mu rhythm with maturation. The concept of “functional topography” (Kuhlman, 1980; Stroganova et al., 1999; Orekhova et al., 2006) suggests that spectral activity in adjacent frequency bins within the same rhythmic component should share similar scalp representations. High-density EEG recordings in the present study provided an opportunity to implement this concept with high-resolution sampling in both spatial and spectral domains to better define age-dependent frequency boundaries of various bands. The K-Means clustering method in Matlab (Arthur and Vassilvitskii, 2007) was used to cluster individual spatio-spectral patterns to different classes with predefined number of classes. Three cluster analyses were performed on spectral topographies (i.e., z scores) of individual frequency bins in the frequency range of 2-9 Hz using data from individual sessions of individual participants, data from averaged sessions at same weekly age point of all participants, and data from averaged sessions at same monthly age point of all participants. Various predefined numbers of classes were explored. Three classes were used as it represented three different frequency bands and nine classes were used with the consideration of three different frequency bands for each of three months of age. Six classes were also tested in order to study the influence of predefined numbers of classes on clustering results.

Based on the clustering results using three classes, frequency boundaries between the delta and theta bands and between the theta and alpha bands were redefined at the resolution of months. Besides spectral patterns, spatial patterns of PSDs in delta, theta, and alpha bands at each month were constructed based on age-dependent boundary definition of frequency bands,
i.e., averaging the topographies from all frequency bins within the age-dependent frequency bands. Also, with redefined frequency boundaries of the infant mu rhythm, spectral peak statistics were similarly re-evaluated at each age point (see Section 2.5). Due to different numbers of frequency bins of the mu rhythm at each age point, an index named peak quantity was calculated by dividing number of frequency bins by the total number of peaks at each age point. Aside from the group-level analysis, the consistency of new band definitions for individual participants and sessions was also tested using a correlation analysis. Monthly averaged spatial maps of redefined delta, theta, and alpha bands were used as templates (9 templates from 3 monthly age points and 3 frequency bands), and correlations between spatial maps of redefined delta, theta, and alpha bands from individual sessions of individual participants and these nine templates were calculated.

2.7 Correlation to crawling acquisition

To investigate the functional correlates of mu rhythm development on crawling skill acquisition, the linear regression analysis on the mu rhythm powers calculated from the age-dependent alpha band from each session of each participant as the function of the corresponding time distance, i.e., number of days, from crawling onset was firstly conducted. The analyses were only performed on data from twelve participants, with one participant excluded due to no reported crawling onset dates. Same analyses were also performed for delta and theta bands for the purpose of comparison. The same regression analysis was also performed on data of individual participants and the slope parameters estimated for various frequency bands were statistically compared (i.e., alpha vs theta, alpha vs delta, and theta vs delta) using the Student t test.

3. Results
3.1 Longitudinal changes of spectral powers

Figure 1(a) provides an up-close observation of changes in relative power densities in the motor cortices along weekly age points at a 1/3 Hz resolution. Each curve represents averaged spectral densities from sessions of the same weekly age point. By comparing spectral powers of individual frequency bin, it shows that, as early as 20 weeks of age, the EEG spectral profiles follow the power-law decay, i.e., spectral powers as a reciprocal of frequency (Miller et al., 2009). It reveals that large fluctuations in spectral profiles are mainly observed in the bands of 3-6 Hz and 6-9 Hz, generally in line with the empirical ranges of the theta and alpha bands observed in infant EEG (Saby and Marshall, 2012). Spectral activity in 6-9 Hz presents a consistently changing trend along weekly age points, from a smooth power decaying profile at 20 weeks with no peak shape, developing to a clear bump shape at 24 weeks, until reaching maximal peak shape of all age points at 31 weeks of age (see the right insert in Figure 1(a)). The figure also shows a shifting pattern of the peak frequency of the alpha band towards higher frequency range. Quantitatively, the total power within 6-9Hz, averaged over all sessions within a month, shows an increasing pattern from month 5 to 7 (Figure 1(b)). Student t tests show the significant increase ($p<0.05$) between month 5 and month 7, while not significant between neighbored months ($p>0.05$ between month 5 and 6, or between month 6 and 7). In addition, alpha band powers present positive correlation with age at a weekly resolution ($r=0.7964$; $p<0.05$). On the other hand, the changing pattern of spectral activity within 3-6 Hz with maturation is not conclusive, with no significant differences between any two monthly age points ($p>0.05$). In particular, the spectral profiles from later weeks (29 and 30 weeks) are similar to the earlier weeks (below 25 weeks).
Figure 1 (a). Weekly spectral profiles of infant EEG from week 20 to week 31. Insets: left: the EEG electrode layout consisting of rejected channels (red dots), pre-selected channels over the motor cortex (black crosses), and the rest (green dots); right: enlarged display of spectral profiles between 6 and 9 Hz. (b). Total powers within 6 and 9 Hz at three different months of age, averaged over session data, and error bars indicate standard deviations.

3.2 Peak frequency statistics

Besides the age-related changes of the spectral profiles, spectral peak distributions at different age points provide another angle in evaluating infant mu rhythm development. Figure 2 presents normalized peak distributions between 2 and 9 Hz at 5, 6 and 7 months of age. For all age points, peak frequencies are mostly located in the range of the empirical theta and alpha bands, with very few peaks in the delta band. It also shows clear separation between the theta and alpha bands for all monthly distributions, and no modal peaks in these monthly distributions are observed in the transition band between theta and alpha bands. Furthermore, in the alpha band, the modal peak frequency of the mu rhythm shifts towards high frequency range by observing its distribution within 6-9Hz, as well as revealed by the means (green dashed lines in Figure 2) of Gaussian fitted curves at different months of age.
Figure 2. Spectral peak distributions summarized at three monthly age points. Red curves depict the Gaussian fitted curves and green dashed lines depict means of the fitted curves.

3.3 Topographies of spectral powers at individual frequency bins

Figure 3 depicts spatial patterns of group-level relative PSDs from each frequency bin between 2 Hz and 9 Hz along weekly age points. Each row shows spectral topographies of one weekly age point and each column shows the different frequency bins. While the power-law decaying pattern of EEG spectra obscures a clear-cut of spatial patterns at different frequency bins, there is a general phenomenon that distinct spatial patterns are present among distant frequency bins and similar patterns in adjacent ones. Meanwhile, smaller variations across different age points are observed, while they are more intriguing in indicating the developments of motor functions.

Figure 4 presents the spatially normalized spectral topographies of different frequency bins along weekly age points. This figure reveals distinct scalp distributions at different
frequency bins, which can be approximately categorized into three classes. The first class mainly resides in the low-frequency range below or around 3 Hz (close to the delta band), with large normalized relative PSDs in the anterior regions for majority of spectral topographies in the range. From about 3 to 6 Hz, similar to the empirical theta band, most spectral topographies present large normalized relative PSDs near posterior sites, which partially reach bilateral central sites. In the empirical alpha band (6-9 Hz), large normalized relative PSDs mostly present in the central areas. Similarly, as in Figure 3, relatively smaller variations can be observed when comparing spatial patterns across different weekly age points from the same frequency than variations across different frequencies.
Figure 3. Spectral topographies of individual frequency bins in the resolution of week.
Figure 4. Normalized spectral topographies of individual frequency bins in the resolution of week. The first box shows topographies in the empirical delta band; the second box for the empirical theta band and the third box for the empirical alpha band.

3.4 Refined definition of infant mu rhythm

Figure 5(a-c) shows the results from the three-class clustering analysis using data from individual sessions (Figure 5(a)), weekly averaged sessions (Figure 5(b)), and monthly averaged sessions (Figure 5(c)). Regardless of averaged or individual sessions and averaged sessions at different temporal resolutions, it is observed that three clusters are mainly separated due to their frequency bands. These are consistent with the classical definitions of delta band (2-3 Hz), theta band (3-6 Hz) and alpha band (6-9 Hz). Such separations of frequency bands are also in line with the observations in Figures 3 and 4. Figure 5 also indicates the shifts of frequency boundaries with maturation between delta and theta and between theta and alpha toward higher frequency ranges. This effect is more evident in the averaged data cases (Figure 5(b-c)) than in the individual session case. Regarding the mu rhythm, data from monthly averaged sessions indicate that the theta/alpha boundary is 6 Hz at month 5 and 6, and 6.33 Hz at month 7, demonstrating the band shifting pattern towards higher frequency ranges, which is consistent with the observations in Figures 1 and 2.

The results of the clustering analysis on data from monthly averaged sessions using different predefined number of classes (i.e., 6 and 9) are shown in Figure 5(d-e). It is clear that more separation in the frequency domain, rather than in the time domain (i.e., different months), is observed due to the enforcement of increased number of classes. It is important to note that data from the frequency range of alpha band at month 5 are actually separated from those at
months 6 and 7, as a new cluster when the predefined number of classes is either 6 or 9, which is different from both delta and theta bands.

Figure 5. Results from the clustering analysis of three classes (coded in three different colors) on (a) EEG data from individual sessions of individual participants; (b) EEG data averaged over sessions and participants from same weeks; (c) EEG data averaged over sessions and participants
from same months; (d) Results from the clustering analysis using 6 classes; and (e) 9 classes on EEG data averaged over sessions and participants from same months. Colors from warm (red) to cold (blue) depict 1st to 3rd class (a-c); 1st to 6th class (d); and 1st to 9th class (e).

With characterized age-related boundary changes between different frequency bands, the ranges of delta, theta, and alpha bands can be defined with shifting changes at different monthly age points. With these redefined ranges, averaged spectral topographies of different bands at different months are evaluated, as shown in Figure 6. In particular, the frequency ranges of the mu rhythm at month 5, 6, and 7 are 6-9 Hz, 6-9Hz, and 6.33-9Hz, respectively. This figure demonstrates that distinct spatial representations exist for the different frequency bands. Delta power mainly focuses in the medial frontal lobe. Theta power is mostly located in the posterior regions, which further extends to bilateral central site. Alpha band power is located in the central cortices, presenting a separated and distributed spatial pattern consisting of areas covering left, medial, and right central sites. At month 5, the alpha powers at both left and right sites are slightly toward posterior areas. At months 6 and 7, the alpha powers at both the left and right sites are more centralized and further extend to the anterior regions and the medial alpha power seems obviously enhanced. With refined frequency ranges of the alpha band at different monthly age points, spectral peak statistics of the mu rhythm were recalculated for three spatial areas (i.e., left, right, and medial central sites) from 5 representative channels (two channels from each lateral region and one from medial region), as shown in Figure 7. It reveals an increasing pattern on the number of spectral peaks in the alpha band from month 5 to 7, and such changes are statistically significant between month 5 and month 7, as well as between month 6 and month 7 ($p<0.05$). Furthermore, the linear trend analysis on data of number of peaks at a weekly resolution presents a positive correlation to age ($r=0.24; p<0.05$).
Figure 6. Spectral topographies of three frequency bands in infants based on their redefined frequency ranges. Rows: delta, theta and alpha bands; Columns: month 5, 6, and 7.

Figure 7. Peak statistics of the mu rhythm summarized at three monthly age points based on its redefined frequency range. Error bars denote standard deviations across different sessions within the same age point.
3.5 Correlation between individual topographies and averaged topographic templates

Figure 8 shows the correlation of spectral topographies of individual sessions at individual frequency bins from individual participants with the nine topographic templates in Figure 6 (3 monthly age points at 3 different frequency bands). For all subplots in the figure, rows represent sessions from all participants in a chronological order, while columns indicate individual frequency bins. Each subplot represents correlation values between individual session data and a topographic template for a pair of age point and frequency point. General consistency of individual topographies within re-defined frequency bands (i.e., delta, theta, and alpha) is observed. The separation between the delta and theta band is clearly indicated in both the first and second rows. The separation of the theta and alpha band is clearer in the second row using topographic templates for the theta band than in the third row using topographic templates for the alpha band. Furthermore, the delta/theta and theta/alpha boundaries shift from low to relatively high frequency values as the infants become older, as similarly indicated in Figure 5.
Figure 8. Correlations between spectral topographies of three frequency bands from EEG data of individual sessions of individual participants and nine topographic templates (see Figure 6).

3.6 Behavioral correlate of infant mu rhythm

Figure 9 presents the scatterplots of delta, theta and alpha band powers for all individual sessions along their corresponding time distances to crawling onsets. It shows that only the alpha band power presents positive and significant developmental change ($r = 0.256; p = 0.018$) as infants are close to crawling onset (Figure 9(c)). Comparing to the alpha band, both delta and theta band powers present no significant correlations to crawling onsets (delta, $r = 0.005; p = 0.962$; theta, $r = -0.131; p = 0.234$). Furthermore, the alpha band shows statistically greater slope parameters in all participants than the delta ($p < 0.05$) and theta ($p < 0.05$) bands, while the difference between the delta band and theta band was not significant ($p > 0.05$).
Figure 9. Scatterplot of delta, theta and alpha band powers with linearly fitted lines as a function of time distance from crawling onsets. (a) delta band; (b) theta band; (c) alpha band.

4. Discussion

The present study investigated the development of the mu rhythm in infant EEG from 5 to 7 months of age. With high resolution in both spatial and temporal domains of EEG recordings and simultaneous characterization of spatio-spectral EEG patterns, the comprehensive evaluation of the infant mu rhythm in the present study included three aspects: spectral characteristics in terms of its frequency peak and range, scalp topographies, and its developmental changes. Our present results revealed detailed information about the formation of the infant mu rhythm and its developmental patterns, some of which is consistent with data from older age groups of infants or children regarding its general tendency toward the frequency range of mu rhythm in adults. The findings complement coarse data available in infants close to this age group in the literature (Marshal et al., 2002; Stroganova et al., 1999; Orekhova et al., 2006). Our data suggest that the formation of the infant mu rhythm is around 6 months at a frequency of about 6 Hz, and it shows distinguishing developmental characteristics and indicates clearly separable spatio-spectral patterns as compared with the theta rhythm. The developmental characteristics of the infant mu rhythm are observed in the formation of its frequency peaks, in the shift of both the frequency peak and band boundary, and in the changes of its scalp...
representations. It is revealed that the infant mu rhythm appears over multiple separated sites in the central area, which resemble somatotopic organization of different body parts seen in adults. The infant mu rhythm also indicates significant correlation \((p < 0.05)\) to the crawling onset, which further supports its functional role in motor skill development and acquisition. All these findings provide valuable data about typical mu rhythm formulation and development during infancy, especially in understanding underlying neural changes of an important milestone of crawling skill acquisition.

4.1 Frequency peak of mu rhythm

The frequency peak in the alpha band is one of key components investigated in most infant EEG studies (Stroganove et al., 1999; Orekhova et al., 2006) and it weighs significantly in the characterization of the infant mu rhythm. However, conclusive evaluation of the formation and shifting patterns of the frequency peak in the infant mu rhythm have not yet been reported, largely due to variations in experimental designs, age ranges studied, and data analysis techniques used in different studies. Furthermore, discrepancy about the spectral origin of the mu rhythm exists. While some argue that the theta activity as its precursor, i.e., the mu rhythm enclosed in the theta band at early stage (Berchicci et al., 2011), others consider the infant mu rhythm to only be within the alpha band (Orekhova et al., 2006; Thorpe et al., 2015; Saby and Marshall, 2012).

The longitudinal EEG data obtained in the present study reveal a continuously growing phenomenon of a bump shape in the alpha band, from none at 20 weeks to a small one at 24 weeks and then to a clear one with a distinct peak around 31 weeks of age (Figure 1(a)). The phenomenon is also supported by the quantitative measure of total power within 6-9 Hz, which shows significant increase from month 5 to month 7 (Figure 1(b)). This progression documents
the formation process of the infant mu rhythm in the age range of 5-7 months and the emergence of the mu rhythm peak around 6 months of age. The number of frequency peaks in the alpha band from single-trial data is the smallest at 5 months and keeps increasing through 6 months and 7 months (Figure 7). This provides collaborative evidence that the formulation of mu peak has already started since 5 months of age and it is strengthened with maturation. Furthermore, both weekly spectral profiles (Figure 1(a)) and monthly peak distribution data (Figure 2) demonstrate the shift of the mu peak frequency towards a high value from 5 to 7 months of age, which is consistent, in terms of tendency, with observations from previous studies with infants/children of different ages (Marshal et al., 2002; Stroganova et al., 1999).

Additionally, the formation of the mu rhythm peak is not accompanied with the transition of modal peaks from the theta to the alpha band, as indicated by the separated peak distributions within both bands and the small number of peaks observed in the transitional region between them (Figure 2). While this presents evidence against the theory of the spectral origin of the mu rhythm starting from the theta band (Berchicci et al., 2011), it is in line with many other studies (Orekhova et al., 2006; Thorpe et al., 2015; Saby and Marshall, 2012) that argue the development of the mu rhythm is a stand-alone process in the alpha band. This is also supported by the distinctive spatial topographies observed for the theta and alpha band (Figure 6), as well as by the clear separation of the theta and alpha bands in the clustering results of the combined spatial and spectral data from infant EEG data (Figure 5).

4.2 Mu band in infant EEG

While most previous studies assume a fixed frequency range for the mu rhythm, e.g., 6-9 Hz (Marshall et al., 2011, 2013; Saby et al., 2012) or 6-8 Hz (Paulus et al., 2012; Fox et al., 2001; Davidson and Fox, 1989), age-dependent change of the entire mu frequency range with
maturation is expected due to the fact that the adult mu range does not entirely cover the infant mu range. Based on the functional topography concept that hypothesizes similar spectral topographies of powers for frequency bins within the same band (Kuhlman, 1980; Stroganova et al., 1999; Orekhova et al., 2006), a clustering analysis was performed in the present study on data at a 1/3 Hz frequency resolution. The results show that the mu frequency range and its spatial topographies both change with maturation. The lower boundary of the mu rhythm (the theta/alpha separation) shifts from 6 Hz at 5 and 6 months to 6.33 Hz at 7 months of age (Figure 5(c)). The shifts of the frequency bands are also evident in data from individual sessions of individual infants (Figure 8). These observations reveal that the shift of the mu rhythm peak is accompanied by an upward shift of the entire mu rhythm band, rather than the expansion of the band, which agrees with some previous findings (Berchicci et al., 2011). It is also consistent with the fact that the adult alpha band (8-12 Hz) and the infant alpha band (6-8Hz) do not overlap. This is also consolidated by the significant and positive correlation \((r=0.256; p<0.05)\) between mu power (based on age-related definition) and crawling onset timing, while no significance \((p>0.05)\) for the other two frequency bands investigated (Figure 9). Therefore, our data suggest the existence of the mu range variation during the motor function maturation that should be taken into account in the investigation of mu rhythm characteristics, e.g. its scalp topographies (Figure 6).

4.3 Cortical representation of mu rhythm

Most previous studies only compare spectral profiles from individual landmark channels at each brain region (Stroganova et al., 1999; Marshall et al., 2002; Orekhova et al., 2006). With the high-density recordings and the simultaneous analysis of high-resolution spectral and spatial information, our study reveals detailed topographies of infant delta, theta and alpha bands and
their age-dependent changes for the first time, to the best of our knowledge, in the age range of 5-7 months (Figure 6). Of the three frequency bands, only the alpha band presents major activity in the central cortices (largely covering the human motor cortex), while the other two frequency bands mainly present activity outside of it. This observation supports the practice of using spectral analysis of different frequency bands in infant EEG (Saby and Marshall, 2012) as in adults since frequency-specific activity provides separable functional information. Moreover, the spectral topographies of different frequency bands are consistent across individual participants and sessions (Figure 8). It is worth noting that spatial distribution of infant mu rhythm power is not dominated by the posterior alpha rhythm as it usually happens in adult resting EEG (Scheeringa et al., 2012). This might be attributed to the fact that the posterior alpha in infants has a distinct developmental trajectory from the central alpha rhythm, i.e., mu rhythm. The former one starts at 3-5 Hz at month 3 and reach 6-7 Hz until the end of the first year (Marshall et al., 2002), which might correspond to the theta rhythm in the data from the present study (no investigation performed).

More importantly, obtaining high-resolution spatial patterns provides additional evidence for the association between the mu rhythm and motor function and/or its development beyond spectral patterns (Smith 1941; Hagne et al., 1973). Our results indicate that the topographic structure of the infant mu rhythm presents a bilateral pattern in both the left and right central site, as well as a pattern in the medial central site (Figure 6). The bilateral pattern is consistent with reported results in a recent study that obtained such topographic patterns indirectly by calculating the ratio of spectral powers at the peaks between 6-8 Hz and 4-6 Hz bands (Thorpe et al., 2015), while such a calculation was not well justified. In our study the pattern at the central medial site is observed for the first time, to the best of our knowledge. The medial central pattern starts to
appear at 5 months of age (but not as strong as lateral central patterns), emerges at 6 months and continues to strengthen to the level of lateral central patterns through 7 months of age, which is temporally aligned with the formulation of the infant mu rhythm, indicating the possible connections between the two. Moreover, based on the homunculus organization of the human motor cortex, the central medial site is associated with low extremities and the central lateral sites are associated with upper extremities and other body parts (Cheyne et al., 1991). The asynchronous development of the mu rhythm between medial and lateral sites suggests the timing separation of functional development of different body parts during the first year of life. The delayed development at the medial central site as compared with the lateral ones could indicate the increasing role of movements played by lower extremities during crawling skill acquisition. Also, stronger activities are presented in mu topographies of all age points at the right central site than the left. It could be caused by unparalleled development of motor skills between dominant and non-dominant upper limbs. Unfortunately, no data are not available in the present study to support this hypothesis and future investigations are required.

4.4 Limitations

Despite the contribution of the present study to the knowledge of infant mu rhythm, there exist some limitations that require effort in future studies. First, the present study focused on the characterization of the infant mu rhythm during resting conditions, while its functional reaction to movement execution, another important aspect of mu rhythm characterization, was not addressed. This requires an additional experimental condition involving well-controlled infant movements, which is lacking in the current experimental design. Secondly, since infants are not able to follow instructions like adults, to acquire noiseless baseline EEG, we adopted a popular visual-attention technique used in many infant developmental studies (Marshall et al., 2002;
Orekhova et al., 2006), which might explain variations in the theta activity in the visual areas observed in the present study (Figures 1 and 7). We assumed an independence of motor processing and sustained visual attention, while such an assumption is still subject to further validation. However, motion artifacts in EEG caused by sudden movements of such as neck and others still largely exist, which were handled by preprocessing steps (e.g., removing bad data segments). Such a strategy worked well in addressing motion artifacts at the cost of fragmentizing continuous EEG data, which in turn confined the analysis resolution of frequency to be low (i.e., 1/3 Hz). Third, the clustering analysis used on spatio-spectral EEG data needed an \textit{a priori} restriction on number of classes, and the three-class configuration in the present study (Figure 5(a-c)) was based on empirical consideration of three frequency bands. Fortunately, the investigation of this parameter using different values (6 and 9 in Figure 5(d-e)) revealed consistent results in general and provided more insights into the different spatial patterns of the infant mu rhythm at different times. Lastly, it is worth noting that the variations in correlations are observed between data from individual sessions/participants and the alpha templates (the last row in Figure 8) are relatively larger than those using the delta and theta templates (the first and second rows in Figure 8). While individual variation should be one of the reasons for the phenomenon, it might also attribute to the fact that the correlation measure is one dimensional and does reserve two-dimensional spatial pattern defined in the electrode domain. Another possible factor contributing to the phenomenon might be voluntary suppression of the infant mu rhythm without actual movements, such as motor imagery, which decreases correlation values of individual alpha patterns against alpha templates. Therefore, the separations between the theta and alpha rhythms obtained using theta templates seem more reliable (the second row in Figure 8).
4.5 Implication to interventional neurorehabilitation technologies

Direct and accurate assessment of results of interventional neurorehabilitation technologies for infants at risk of developmental motor disabilities (e.g., CP) is paramount. Understanding neural correlates of changes in movement learning or skill acquisition will not only provide information about the effectiveness of interventions, but also could serve as a feedback to improve the delivery of interventions. In the present study, association of the infant mu rhythm and motor development is derived from a detailed spatio-spectral analysis on the developmental changes of mu rhythm’s peak, frequency range, and topographic representations, as well as directly from the significant correlation between infant mu rhythm power and crawling onset timing. Our results suggest that the mu rhythm is a potential neural biomarker for such assessments. Further studies with infants at the risk of developmental motor disabilities are needed to investigate its role in assessing interventional technologies for neurorehabilitation.

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Highlights
- Data driven methods reveal developmental changes of mu rhythm in 5-7 month infants.
- Infant mu rhythm is suggested to formulate around 6 months at about 6 Hz.
- Its developmental features are also observed in its frequency peak and band shifts.
- It shows maturation at multiple central areas, resembling somatotopic organization.
- Power of infant mu rhythm indicates a significant correlation to the crawling onset.